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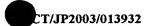


(54) Title: NUCLEIC ACIDS ISOLATED FROM NEUROBLASTOMA AT STAGE 4S

(54)発明の名称: 4 s 期神経芽細胞腫から単離された核酸

(57) Abstract: Prognosis (in particular, determination of the progress stage and judgment of neuroblastoma at stage 4s) of neuroblastoma is diagnosed by using a diagnostic agent for the prognosis of neuroblastoma and a diagnostic kit comprising a nucleic acid probe, a primer or a nucleic acid microarry with the use of a nucleic acid comprising a sequence selected from among the nucleic acid sequences represented by SEQ ID NOS:1 to 174, fragments thereof and combinations of the same.

(57)要約: 配列表の配列番号 1 ないし 1 7 4 に記載の核酸配列からなる群より選ばれる 1 つの配列からなる核酸 若しくはその断片等、或いはその組み合わせを利用した核酸プローブ、プライマーまたは核酸マイクロアレイから なる、神経神経芽細胞腫の予後診断剤および診断キットを用いて、神経芽細胞腫の予後(特に、進行度分類および 4 s 期神経芽細胞腫の判定)を診断する。



明細書

4 s 期神経芽細胞腫から単離された核酸

技術分野

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【0001】 本発明は、ヒト神経芽細胞腫において発現する遺伝子に由来する核酸類に関する。さらに詳しくは、本発明は、4 s 期のヒト神経芽細胞腫において発現する遺伝子に由来する核酸類に関する。さらに、本発明は、このような核酸およびそれらの断片、あるいはそれらの組み合わせを利用した核酸プローブ、プライマーまたは核酸マイクロアレイ等からなる、4 s 期神経芽細胞腫の診断剤および診断キット、さらには上記遺伝子からの核酸配列情報に基づく癌細胞のプログラム細胞死機構の解明に関する。

背景技術

【0002】 (腫瘍形成と遺伝子)

個々の腫瘍にはそれぞれの個性があり、発癌の基本的な原理は同じであっても、 その生物学的特性は必ずしも同じではない。近年、癌の分子生物学や分子遺伝学 が急速に進歩し、発癌やいわゆる腫瘍細胞のバイオロジーが遺伝子レベルで説明 できるようになってきた。

【0003】 (神経芽細胞腫)

神経芽細胞腫は、末梢交感神経系細胞に由来する交感神経節細胞と副腎髄質細胞から発生する小児癌である。この交感神経系細胞は、発生初期の神経堤細胞が腹側へ遊走し、いわゆる交感神経節が形成される場所で分化成熟したものである。その一部の細胞はさらに副腎部へ遊走し、先に形成されつつある副腎皮質を貫通して髄質部に達し、そこで髄質を形成する。神経堤細胞は、ほかの末梢神経細胞の起源ともなっており、後根神経節(知覚神経)、皮膚の色素細胞、甲状腺C細胞、肺細胞の一部、腸管神経節細胞などへ分化する。

【0004】 (神経芽細胞腫の予後)

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神経芽細胞腫は多彩な臨床像を示すことが特徴である(中川原,「神経芽腫の発生とその分子機構」,小児内科,1998年,第30巻,p.143)。例えば、1歳未満で発症する神経芽細胞腫は非常に予後が良く、大部分が分化や細胞死を起こして自然退縮する(予後良好型ともいう)。現在、広く実施されている生後6か月時の尿のマススクリーニングで陽性となる神経芽細胞腫の多くは、この自然退縮を起こしやすいものに属する。一方、1歳以上で発症する神経芽細胞腫は悪性度が高く、多くの場合、治療に抵抗して患児を死に至らしめる(予後不良型ともいう)。1歳以上の悪性度の高い神経芽細胞腫は、体細胞突然変異

(Somatic mutation) が起こり、モノクローナルであるのに対し、自然退縮する神経芽細胞腫では生殖細胞突然変異 (germ line mutation) のみの遺伝子変異でとどまっているとの仮説もある (Knudson AG et al., Regression of neuroblastoma IV-S:A genetic hypothesis N. Engl. J. Med., U.S.A. 1980, vol 302, p. 1254)。さらに、臨床的にこれらの型の中間に位置する中間型の神経芽細胞腫もある。

15 【0005】 腫瘍の進行度からこれら神経芽細胞腫を分類すると以下のように なる。

1期:副腎または交感神経節に原発し、限局している。

2期:原発巣に限局した腫瘍と局部リンパ節転移のみを有する。リンパ節転移は 正中線を越えない。

20 3期:腫瘍が正中線を越えて対側に浸潤またはリンパ節転移をきたす。

4期:骨、骨髄、眼窩部に遠隔転移を起こす。

4 s 期:1歳未満に発症し、骨髄、皮膚、肝に遠隔転移する。

【0006】 予後良好型の神経芽細胞腫は、1、2、4 s 期の腫瘍であり、予後不良型および中間型の神経芽細胞腫は、3、4 期の腫瘍である。4 s 期の腫瘍は、特異的であり、通常生後数ヶ月の乳児に発症し、急速に腫瘍が増殖転移するが、突然増殖が止まり、その後は自然に腫瘍が消失する。このように、自然退縮

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する腫瘍と悪性増殖する腫瘍との間の違いは、発症年齢と転移部位、さらに進行 度が明らかに異なる。

【0007】 (神経芽細胞腫の予後を推定する遺伝子)

最近の分子生物学的研究の進展により、神経成長因子 (nerve growth factor: NGF) の高親和性レセプターであるTrkAの発現が分化と細胞死の制御に深くかかわっていることが明らかとなってきた (Nakagawara A., The NGF story and neuroblastoma, Med. Pediatr. Oncol., U.S.A., 1998, Vol 31, p. 113)。 Trkは神経栄養因子の高親和性受容体で、膜貫通型受容体であり、Trk-A、B、Cの3つが主なものである。

【0008】 Trkファミリー受容体は、中枢神経および末梢神経系において、特異的な神経細胞の分化と生存維持に重要な役割を果たしている(中川原等,「神経芽細胞腫におけるニューロトロフィン受容体の発現と予後」,小児外科,1997年,第29巻,p.425-432)。腫瘍細胞の生存や分化はTrkチロシンキナーゼやRetチロシンキナーゼからのシグナルで制御されている。なかでも、TrkA受容体の役割は最も重要で、予後良好型の神経芽細胞腫ではTrkAの発現が著しく高く、これからのシグナルが腫瘍細胞の生存・分化、または細胞死(アポトーシス)を強く制御している。一方、予後不良型の神経芽細胞腫では、TrkAの発現が著しく抑えられており、これに代わってTrkBあるいはRetからのシグナルが生存の促進という形で腫瘍の進展を助長している。

【0009】 また、神経の癌遺伝子であるN-mycの増幅が神経芽細胞腫の予後に関連していることも明らかになってきた(中川原,「脳・神経腫瘍の多段階発癌」, Molecular Medicine, 1999年, 第364巻, p. 366)。この遺伝子は神経芽細胞腫で初めてクローニングされたが、正常細胞や予後良好型の神経芽細胞腫では通常1倍体当たり1つしか存在しないのに対し、予後不良型の神経芽細胞腫においては数十倍に増幅されているのが見つかった。



【0010】 上記の遺伝子以外にも、予後良好型の神経芽細胞腫で高発現する遺伝子として、CD44、PTN、caspase 等が知られており、また予後不良型の神経芽細胞腫で高発現する遺伝子としては、SVV (survivin)、MK (midkine) 等が知られている。

5 【0011】 さらに、本発明者らは、予後良好型の神経芽細胞腫において、一 群の新規な遺伝子が高発現していることを見出し(国際公開PCT/JP016 31号パンフレット)、また対照的に予後不良型の神経芽細胞腫において、別の 一群の新規な遺伝子が高発現していることを見出した(国際公開PCT/JP0 1629号パンフレット)。

10 【0012】 しかしながら、現在までに4s期神経芽細胞腫において発現する (特に、特異的に)遺伝子についてはほとんど知られていなかった。さらに、上記のように4s期神経芽細胞腫は自然退縮するので、この原因となる遺伝子の同定も急務である。

発明の開示

- 15 【0013】 本発明は、上記従来技術の有する課題に鑑みてなされたものであり、一般的に神経芽細胞腫の予後良不良に関係する遺伝子の核酸配列を明らかにし、そのような遺伝子情報の提供および予後良不良に関する診断を可能とすることを目的とする。本発明は、特定的には神経芽細胞腫の予後を診断し、該細胞腫の進行度分類を行い、4 s 期神経芽細胞腫の判定を可能とすることを目的とする。
- 20 【0014】 本発明者らは鋭意研究した結果、ヒト神経芽細胞腫の予後を検定し、予後良好型および予後不良型の臨床組織の各々からcDNAライブラリーを作製することに成功した。これら2種類のcDNAライブラリーから各々約2400個のクローンをクローニングし、神経芽細胞腫の予後の良悪によって分類し、それぞれのサブセットで遺伝子のプロファイリングを行った。
- 25 【0015】 そこで本発明者らは、前記サブセット間で示差的に発現し、かつ 予後良好型の臨床組織でのみ発現が増強している遺伝子群を見いだした。加えて、

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本発明者は、予後不良型の臨床組織でのみ発現が増強している遺伝子群をも見いだした。かかる知見に基づき、本発明者は少なくとも予後良好型の臨床組織または、予後不良型の臨床組織でのみ発現が増強している遺伝子を検出およびクローニングするための核酸配列情報を提供することを可能とした。

【0016】 さらに、本発明者らは、4s期神経芽細胞腫の臨床組織から同様にcDNAライブラリーを作製することに成功した。このライブラリーから約2700個のクローンをクローニングした。このライブラリーのサブセットと、予後良好型および予後不良型の臨床組織からのライブラリーのサブセットを解析して、これらのサブセット間で発現する約1600個の遺伝子のプロファイリングを行った。その結果、前記サブセット間で示差的に発現する452個の遺伝子を同定した。これらの遺伝子をシークエンスしたところ、308個の新規な遺伝子と、残り144個の既知の遺伝子とから成っていた。前記遺伝子をそれぞれのサブセット間での発現パターンに従って、分類し7つの群にグループ化した。

【0017】 かかる知見に基づき、本発明者らは、4 s 期神経芽細胞腫を特徴づける発現パターンを呈する遺伝子を検出およびクローニングするための遺伝子情報(核酸配列情報等)を提供することを可能とした。さらに該核酸配列情報に基づき、神経芽細胞腫の予後診断法(特に、進行度分類)を、4 s 期神経芽細胞腫の判定を含めて、可能とする診断剤や診断キットを提供することを可能とし、本発明を完成した。

【0018】 すなわち、本発明によれば、配列表の配列番号1ないし174に 記載の核酸配列からなる群より選ばれる1つの配列からなる核酸が提供される。

【0019】 好ましい核酸は、前記配列番号1ないし174のうち、配列番号 1ないし14のいずれか一つに記載の核酸配列からなる核酸である。

【0020】 また、本発明によれば、上記これらの核酸に相補的な核酸も提供される。

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【0021】 また、本発明によれば、上記の核酸と、またはそれに相補的な核酸とストリンジェントな条件下でハイブリダイズする核酸が提供される。

【0022】 また、本発明によれば、以下の(a)或いは(b)の核酸を含む核酸プロープが提供される:

- 5 (a)配列表の配列番号1ないし174に記載の核酸配列からなる群より選ばれる 1つの配列の全長若しくは一部からなる核酸、またはそれに相補的な核酸:
 - (b)配列表の配列番号1ないし174に記載の核酸配列からなる群より選ばれる 1つの配列からなる核酸とストリンジェントな条件下でハイブリダイズする核酸、 またはそれに相補的な核酸。
- 10 【0023】 好ましくは、前記(a)或いは(b)の核酸がDNAである。
 - 【0024】 また、好ましくは、前記(a)または(b)の核酸が配列番号1ないし14に記載の核酸配列からなる群より選ばれる1つの配列からなる核酸である。
 - 【0025】 また、本発明によれば上記の核酸プローブを有効成分として含有する4s期神経芽細胞腫の診断剤が提供される。
- 15 【0026】 さらに、本発明によれば、以下の(a)或いは(b)のDNAを含むプライマーが提供される:
 - (a)配列表の配列番号175ないし1076に記載の核酸配列からなる群より選ばれる1つの配列からなるDNA、またはそれに相補的なDNA;
 - (b)配列表の配列番号175ないし1076に記載の核酸配列からなる群より選ばれる1つの配列からなるDNAとストリンジェントな条件下でハイブリダイズするDNA、またはそれに相補的なDNA。
 - 【0027】 好ましくは、前記(a)或いは(b)のDNAが配列番号175ないし202に記載の核酸配列、および配列番号519ないし540に記載の核酸配列からなる群より選ばれる1つの配列からなるDNA、または配列表の配列番号785ないし798に記載の核酸配列からなる群より選ばれる1つの配列からなるDNAである。

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【0028】 また、本発明によれば上記のプライマーを一組、有効成分として 含有する4 s 期神経芽細胞腫の診断キットが提供される。

【0029】 また、本発明によれば神経芽細胞腫の臨床組織サンプルから配列 表の配列番号1ないし14に記載の核酸配列からなる群より選ばれる1つの配列 からなる核酸の有無を検出することを特徴とする、4s期神経芽細胞腫の判定方法が提供される。

【0030】 加えて、本発明によれば固相支持体に、配列表の配列番号1ない し174に記載の核酸配列からなる核酸の全長若しくは一部からなる核酸を複数 個組み合わせて、固定してなる核酸マイクロアレイが提供される。

【0031】 また、本発明によれば固相支持体に、配列番号175ないし202に記載の核酸配列、配列番号519ないし540に記載の核酸配列、および配列番号785ないし798に記載の核酸配列からなる核酸を複数個組み合わせ、それらを固定してなる核酸マイクロアレイが提供される。ここで、記載された配列番号を有する核酸配列からなる核酸の複数個の任意の組み合わせが用いられる。発明を実施するための最良の形態

【0032】 以下、本発明に係る神経芽細胞腫に発現する遺伝子(以下、「本発明の遺伝子」という)に由来する核酸(以下、「本発明の核酸」という)について、その用途を含めて、本発明の好適な実施の形態を参照して、詳細に説明する。

【0033】 本発明の核酸は、上述のごとく本発明の遺伝子に由来するものであり、該遺伝子を構成するか或いは該遺伝子からインビボまたはインビトロの過程によって得られる。該核酸の鎖長には特に制限はなく、本明細書では前記遺伝子の一部に対応する核酸断片を含めて「本発明の核酸」という。核酸の鎖長が短い場合、その核酸は化学的手法で合成することができる。

【0034】 本明細書で使用する「核酸」という用語は、例えばDNAまたは RNA、或いはそれらから誘導された活性なDNA若しくはRNAでありうるポ

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リヌクレオチドを指し、好ましくは、DNAまたはRNAを意味する。特に好ましい核酸は、本明細書中に開示されるヒトcDNA配列と同一か、またはそれに相補的な配列を有する。

【0035】 また、本発明で使用する「ストリンジェントな条件下でハイブリダイズする」という用語は、2つの核酸(または断片)が、Sambrook, J., Expression of cloned genes in E. coli, Molecular Cloning: A laboratory manual, U.S.A., Cold Spring Harbor Laboratory Press, 1989, p. 9. 47-9. 62, p. 11. 45-11. 61 に記載されたハイブリダイゼーション条件下で、相互にハイブリダイズすることを意味する。

【0036】 より具体的には、前記「ストリンジェントな条件」とは、約45℃において $6.0 \times SSC$ でハイブリダイゼーションを行った後に、50℃で $2.0 \times SSC$ で洗浄することを指す。ストリンジェンシーの選択のため、洗浄工程における塩濃度を、例えば低ストリンジェンシーとしての約 $2.0 \times SSC$ 、50℃から、高ストリンジェンシーとしての約 $0.2 \times SSC$ 、50℃まで選択することができる。さらに、洗浄工程の温度を低ストリンジェエンシー条件の室温、約22℃から、高ストリンジェンシー条件の約65℃まで高くすることができる。

【0037】 また、本明細書で使用する「核酸」という用語は、単離された核酸を指し、これは組換之DNA技術により調製された場合は細胞物質、培養培地を実質的に含有せず、化学合成された場合には前駆体化学物質またはその他の化学物質を実質的に含まない、核酸またはポリペプチドを指す。

【0038】 本明細書で使用する「予後良好型」とは、ヒト神経芽細胞腫のうち、腫瘍が限局して存在するか、または退縮や良性の交感神経節細胞腫になった状態を指し、N-mycその他腫瘍マーカー(TrkA、染色体異常等)から判断して、悪性度が低いと医師によって判断されるものである。本発明の好適な実施の形態では、病期1または2、発症年齢が1歳未満、手術後5年以上再発なく生存し、臨床組織中にN-mycの増幅が認められないものを予後良好型とした

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が、このような特定の例には限定されない。また、本明細書で使用する「予後不良型」とは、ヒト神経芽細胞腫のうち、腫瘍の進行が認められる状態を指し、N-mycその他腫瘍マーカーから判断して、悪性度が高いと医師によって判断されるものである。本発明の好適な実施の形態では、病期 4、発症年齢が 1 歳以上、手術後 3 年以内に死亡、臨床組織中にN-mycの増幅が認められたものを予後不良型としたが、このような特定の例には限定されない。

【0039】 なお、4s期神経芽細胞腫は、上記のような臨床分子生物学的分類に従えば「予後良好型」に分類されるが、本明細書中では便宜上、「予後良好型」とは区別して取り扱う。

【0040】 神経芽細胞腫は、ヒトでは2種類しか知られていない神経細胞そのものの腫瘍の1つであり、そこで発現している遺伝子を解析することは、神経細胞のバイオロジーを理解する上で非常に有用な知見をもたらすものと考えられる。すなわち、脳や末梢神経から、部位特異的な均質な組織を得ることは極めて困難で、事実上不可能である。一方、神経芽細胞腫は、末梢交感神経細胞に由来するほぼ均一な神経細胞集団(腫瘍化してはいるが)から成り、均質に発現している神経関連遺伝子が得られる可能性が高い。また、神経芽細胞腫は癌であるため、神経発生の未熟な段階で発現している重要な遺伝子が多いことも特徴として挙げられる。

【0041】 さらに、神経芽細胞腫は、予後の良好なものと予後の不良なものとが臨床的、生物学的に明瞭に区別される。予後良好型の神経芽細胞腫の癌細胞は、増殖速度が極めて遅く、ある時点から自然退縮を始めることが特徴である。これまでの知見から、この自然退縮では、神経細胞の分化およびアポトーシス(神経細胞死)が起こっており、正常神経細胞の成熟段階で起こる分化とプログラム細胞死と非常によく似た現象であることが分かってきた。従って、この腫瘍で発現している遺伝子を解析することによって、神経の分化やアポトーシスに関連した重要な遺伝子情報を入手できる可能性が極めて高い。

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【0042】 上記の有用な遺伝子情報を入手できる遺伝子である本発明の遺伝子およびそれらに由来する本発明の核酸は、4 s 期神経芽細胞腫の臨床組織(以下、4 s とも略称する)に見出されたものであるが、予後良好型の臨床組織(以下、"F (favorable)"とも略称する)および予後不良型の臨床組織(以下、"UF (unfavorable)"とも略称する)でのそれら遺伝子の発現を比較すると以下のような特徴を有する。

【0043】 すなわち、前述のようにして得られ、少なくとも部分的にシーク エンスした452個の遺伝子をそれぞれのサブセット間での発現パターンに基づ いて、分類し7つの群にグループ化したところ、次のようになる。

【0044】 (グループI)

このグループに属する遺伝子は、その発現(4s)がUFと同程度であり、Fより低い。さらに、これら遺伝子をサブグループに分類すると、I-1、I-2およびI-3となる。各サブグループの遺伝子発現パターンについては、表1を参照。

15 【0045】 I-1に属する特定のクローンは、nbla20026(配列番号 171), nbla20421(配列番号 172), nbla22298(配列番号 173), nbla22549(配列番号 174) および nbla23020(以上、新規遺伝子)である。

【0046】 I-2に属する特定のクローンは、nbla20113, nbla20146(配列番号 137), nbla20170(配列番号 138), nbla20216(配列番号 139), nbla20253, nbla20549, nbla20657(配列番号 140), nbla20688(配列番号 141), nbla20755(配列番号 142), nbla20835, nbla20968, nbla21013(配列番号 143), nbla21087, nbla21172(配列番号 144), nbla21189, nbla21200(配列番号 145), nbla21214, nbla21255(配列番号 146), nbla21337, nbla21344, nbla21345(配列番号 147), nbla21410(配列番号 148), nbla21522(配列番号 149), nbla21631(配列番号 150), nbla21788(配列番号 151), nbla21897(配列番号 152), nbla21956, nbla22116(配列番号 153), nbla22223(配列番号 154), nbla22228, nbla22344(配列番号 155),

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nbla22351, nbla22361, nbla22474, nbla22629, nbla22939(配列番号 156), nbla23084(配列番号 157), nbla23103(配列番号 158), nbla23234(配列番号 159), nbla23300(配列番号 160), nbla23369(配列番号 161), nbla23436(配列番号 162), nbla23511(配列番号 163), nbla23664(配列番号 164), nbla23775, nbla23860(配列番号 165), nbla23877(配列番号 166), nbla23998(配列番号 167), nbla24043(配列番号 168), nbla24182, nbla24285, nbla23998(配列番号 169), nbla24043(配列番号 168), nbla24762, nbla24285, nbla24402(配列番号 169), nbla24434, nbla24460, nbla24762, nbla24821(配列番号 170), nbla24893, nbla24973, nbla24986(以上、新規遺伝子)、nbla20279, nbla20687, nbla20924, nbla21168, nbla21303, nbla21483, nbla21838, nbla21917, nbla22099, nbla22438, nbla23111, nbla23208, nbla24118, nbla24279, nbla24771および nbla24871(以上、既知遺伝子)である。

【0047】 I-3に属する特定のクローンは、nbla20084(配列番号 129), nbla21081(配列番号 130), nbla21420(配列番号 131), nbla21761, nbla22452(配列番号 132), nbla22595(配列番号 133), nbla22676(配列番号 134), nbla22909(配列番号 135), nbla23456, nbla24297, nbla24435(配列番号 136), nbla24719(以上、新規遺伝子)、nbla20117, nbla20238, nbla20904, nbla23293, nbla23297, nbla23311, nbla23589, nbla23629, nbla23862, nbla24133およびnbla24761(以上、既知遺伝子)である。

【0048】 (グループ II)

このグループに属する遺伝子は、その発現(4 s)がFと同程度であり、UFより高い。さらに、これら遺伝子をサブグループに分類すると、II-1、II-2 および II-3となる。各サブグループの遺伝子発現パターンについては、表1を参照。

【0049】 II-1に属する特定のクローンは、nbla20365(配列番号 117), nbla20378(配列番号 118), nbla20511(配列番号 119), nbla21039(配列番号 120), nbla21107(配列番号 121), nbla21367(配列番号 122), nbla21790(配列番号 123),

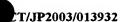
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nbla21855, nbla22253(配列番号 124), nbla22355(配列番号 125), nbla22704, nbla22832(配列番号 126),nbla23394,nbla23512,nbla23755(配列番号 127), nbla24084, nbla24376, nbla24549(配列番号 128)(以上、新規遺伝子)、nbla20624, nbla22029, nbla22424, nbla22594 および nbla22622 (以上、既知遺伝子) である。 II-2に属する特定のクローンは、nbla20001(配列番号 58), nbla20083(配列番号 59), nbla20125, nbla20182(配列番号 60), nbla20231, nbla20248(配列番号 61),nbla20250(配列番号 62),nbla20268,nbla20330(配列 番号63), nbla20395, nbla23973, nbla23983(配列番号64), nbla24041, nbla24082, nbla24104, nbla24111(配列番号 65), nbla24142(配列番号 66), nbla24157(配列 番号 67), nbla24230(配列番号 68), nbla24239, nbla20541(配列番号 69), nbla20555(配列番号 70), nbla20638, nbla20645(配列番号 71), nbla20713(配列 番号 72), nbla20765, nbla20789, nbla20792, nbla20798, nbla21024, nbla24250(配列番号 73),nbla24254(配列番号 74),nbla24327(配列番号 75), nbla24363, nbla24510(配列番号 76), nbla24554(配列番号 77), nbla24604(配列 番号78), nbla24622, nbla24646, nbla24672, nbla21037(配列番号79), nbla21077, nbla21089, nbla21130, nbla21161(配列番号80), nbla21170(配列番号81), nbla21198(配列番号 82),nbla21266,nbla21298(配列番号 83),nbla21379(配列 番号84), nbla24705(配列番号85), nbla24709, nbla24748, nbla24831, nbla24972, nbla21385(配列番号 86),nbla21413,nbla21416(配列番号 87),nbla21520, nbla21599(配列番号 88),nbla21681(配列番号 89),nbla21878(配列番号 90), nbla21922(配列番号91), nbla21936, nbla22004-2(配列番号92), nbla22004-1(配 列番号 93), nbla22028, nbla22085(配列番号 94), nbla22093, nbla22119(配列番 号 95), nbla22149 (配列番号 96), nbla22161 (配列番号 97), nbla22218, nbla22252(配列番号 98),nbla22347(配列番号 99),nbla22352(配列番号 100), nbla22394(配列番号 101), nbla22423(配列番号 102), nbla22439(配列番号 103), nbla22451, nbla22455, nbla22464, nbla22465, nbla22487, nbla22633(配列番号



104), nbla22669, nbla22698(配列番号 105), nbla22726, nbla22886, nbla22896(配列番号 106), nbla23012, nbla23038, nbla23167(配列番号 107), nbla23339(配列番号 108), nbla23352(配列番号 109), nbla23575(配列番号 110), 23592(配列番号 111), nbla23601(配列番号 112), nbla23630(配列番号 113), nbla23718, nbla23719, nbla23754(配列番号 114), nbla23892(配列番号 115), nbla23951, nbla23956(配列番号 116)(以上、新規遺伝子)、nbla20393, nbla20423, nbla20510, nbla20833, nbla20931, nbla20943, nbla21258, nbla21268, nbla21273, nbla21412, nbla21578, nbla21614, nbla21624, nbla21655, nbla21670, nbla21787, nbla21954, nbla21979, nbla22043, nbla22137, nbla22192, nbla22325, nbla22327, nbla22337, nbla22482, nbla22763, nbla22788, nbla22839, nbla22851, nbla22935, nbla22937, nbla23384, nbla23812, nbla23823, nbla23849, nbla23882, nbla23910, nbla24064, nbla24405, nbla24897 および nbla24913 (以上、既知遺伝子)である。

【0051】 II-3に属する特定のクローンは、nbla20134, nbla20181, nbla20264(配列番号 31), nbla20269(配列番号 32), nbla20276, nbla20406(配列番号 33), nbla20709, nbla20782, nbla20788, nbla20949(配列番号 34), nbla21046, nbla21122, nbla21211, nbla21233, nbla21251(配列番号 35), nbla21334(配列番号 36), nbla21356(配列番号 37), nbla21375, nbla21418(配列番号 38), nbla21480(配列番号 39), nbla21509(配列番号 40), nbla21524, nbla21527(配列番号 41), nbla21551(配列番号 42), nbla21735(配列番号 43), nbla21843, nbla21934, nbla22153, nbla22247(配列番号 44), nbla22382, nbla22477(配列番号 45), nbla22571, nbla22639(配列番号 46), nbla22789, nbla23060, nbla23174(配列番号 47), nbla23198(配列番号 48), nbla23218, nbla23328(配列番号 49), nbla23420(配列番号 50), nbla23483(配列番号 51), nbla23545, nbla23653, nbla23666, nbla23760, nbla23808(配列番号 52), nbla23830, nbla23851(配列番号 53), nbla23942, nbla24011(配列番号 54), nbla24131,

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nbla24235(配列番号 55), nbla24556(配列番号 56), nbla24800(配列番号 57), nbla24908(以上、新規遺伝子)、nbla20133, nbla20263, nbla20723, nbla20748, nbla20915, nbla21016, nbla21034, nbla21067, nbla21167, nbla21319, nbla21331, nbla21516, nbla21682, nbla21691, nbla21822, nbla21976-2, nbla21977, nbla22159, nbla22168, 22215-1, nbla22244, nbla22263, nbla22548, nbla23033, nbla23231, nbla23284, nbla23329-1, nbla23384, nbla23556, nbla23674, nbla23879-2, nbla24098, nbla24329, nbla24334, nbla24439-1, nbla24443, nbla24507, nbla24836, nbla24958 およびnbla24989(以上、既知遺伝子)である。

【0052】 (グループ III)

このグループに属する遺伝子は、その発現(4s)がFと同程度であり、UFより低い。さらに、これら遺伝子をサブグループに分類すると、III-1、III-2およびIII-3となる。各サブグループの遺伝子発現パターンについては、表1を参照。

【0053】 III-1に属する特定のクローンは、nbla20874(新規遺伝子) および nbla23262 (既知遺伝子) である。

【0054】 III-2に属する特定のクローンは、nbla20604, nbla21226, nbla21908(配列番号 27), nbla21928, nbla22027(配列番号 28), nbla22082(配列番号 29), nbla22643, nbla23303(配列番号 30), nbla23649, nbla24468(以上、新規遺伝子)、nbla20141, nbla20446, nbla21538, nbla21558, nbla21623, nbla21969, nbla22219, nbla23272, nbla23307およびnbla24117(以上、既知遺伝子)である。

【0055】 III-3に属する特定のクローンは、nbla20578(配列番号 26), nbla21212(以上、新規遺伝子)、nbla23478, nbla23896 およびnbla24920(以上、既知遺伝子) である。

25 【0056】 (グループ IV)

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このグループに属する遺伝子は、その発現(4s)がUFと同程度であり、Fより高い(F<4s=UF)。このグループに属する特定のクローンは、nbla23899(配列番号 25)および nbla24526 (以上、新規遺伝子) である。

【0057】 (グループV)

5 このグループに属する遺伝子は、その発現(4s)がFより低く、UFより高い。さらに、これら遺伝子をサブグループに分類すると、V-1、V-2、V-3、V-4およびV-5となる。各サプグループの遺伝子発現パターンについては、表1を参照。

【0058】 V-1に属する特定のクローンは、nbla22031 (既知) である。V-2に属する特定のクローンは、nbla22305 (既知) である。

【0059】 V-3に属する特定のクローンは、nbla20123(配列番号 17), nbla20382(配列番号 18), nbla20660(配列番号 19), nbla20666(配列番号 20), nbla21239(配列番号 21), nbla21729(配列番号 22), nbla21831(配列番号 23), nbla22826(配列番号 24), nbla24521(以上、新規遺伝子)、nbla20235 および nbla22607(以上、既知遺伝子)である。

【0060】 V-4に属する特定のクローンは、nbla20787(配列番号 15), nbla22284(配列番号 16)およびnbla24756(以上、新規遺伝子)である。

【0061】 V-5に属する特定のクローンは、nbla24348 および nbla24686 (以上、新規遺伝子) である。

20 【0062】 (グループ VI)

このグループに属する遺伝子は、その発現(4s)がFおよびUFより低いか、またはFおよびUFより高い。さらに、これら遺伝子をサブグループに分類すると、VI-1、VI-2、VI-3、VI-4、VI-5、VI-6、VI-7およびVI-8となる。各サブグループの遺伝子発現パターンについては、表 1 を参照。

25 【0063】 VI-1に属する特定のクローンは、nbla21297(配列番号 14) (新 規遺伝子) および nbla22443 (既知遺伝子) である。



【0064】 VI-2に属する特定のクローンは、nbla20211, nbla20469, nbla21250, nbla22182 (配列番号 12), nbla22761, nbla23256 (配列番号 13), nbla23631, nbla23711, nbla24532, nbla24951 (以上、新規遺伝子)、nbla21750, nbla22129, nbla22808, nbla23064 およびnbla23358 (以上、既知遺伝子) である。

5 【0065】 VI-3に属する特定のクローンは、nbla20226(配列番号11)(新 規遺伝子)である。

【0066】 VI-4に属する特定のクローンは、nbla21650(配列番号7), nbla22094(配列番号8), nbla22739(配列番号9)および nbla23525(配列番号10) (以上、新規遺伝子) である。

10 【0067】 VI-5に属する特定のクローンは、nbla23701(配列番号5)および nbla23890(配列番号6)(以上、新規遺伝子)である。

【0068】 VI-6に属する特定のクローンは、nbla20087(既知遺伝子)である。

【0069】 VI-7に属する特定のクローンは、nbla22689(配列番号2),

15 nbla22968, nbla24079, nbla24135(配列番号 3)および nbla24350(配列番号 4)(以上、新規遺伝子)である。

【0070】 VI-8に属する特定のクローンは、nb1a22256(新規遺伝子)である。

【0071】 (グループ VII)

20 このグループに属する遺伝子(1個のみ)は、4 s でのみ発現している。その 特定のクローンは、nbla22420(配列番号 1) (新規遺伝子) である。

【0072】 前記それぞれのグループについて、遺伝子群を新規な遺伝子と、 既知の遺伝子に分け、まとめたものが表1である。

25 【0073】 表1



グループ	発現パターン	新規遺伝子	既知遺伝子	計
1-1	F≫4s≓UF	5	0	5
1-2	F>4s=UF	59	16	75
I -3	F24s=UF	12	11	23
11-1	F=4s≫UF	18	5	23
11-2	F=4s>UF	105	47	152
11-3	F=4s≥UF	55	40	95
111-1	F=4s≪UF	1	1	2
111-2	F=4s <uf< td=""><td>10</td><td>10</td><td>20</td></uf<>	10	10	20
111-3	F=4s <uf< td=""><td>2</td><td>3</td><td>5</td></uf<>	2	3	5
IV	F<4s=UF	2	0	2
V-1	F>4s≫UF	0	1	1
V-2	F≥4s≫UF	0	1	1
V-3	F>4s>UF	9	2	11
V-4	F≥4s>UF	3	0	3
V-5	F≥4s≥UF	2	0	2
VI-1	F>>4s <uf< td=""><td>1</td><td>1</td><td>2</td></uf<>	1	1	2
V1-2	F>4s(UF	10	5	15
VI-3	F>4s≤UF	1	0	1
VI-4	F≥4s≤UF	4	0	4
VI-5	F<4s≫UF	2	0	2
V1-6	F≤4s≫UF	0	1	1
VI-7	F<4s>UF	5	0	5
V1-8	F≤4s≥UF	1	0	1
VII	4s のみ	1	0	1
クローン総数		308	144	452

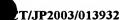
なお、表中および上記分類において、「=」は遺伝子発現量がサブセット間で

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ほぼ等しいことを示す。

【0074】 例えば、グループ VI に属する遺伝子群は、4 s 期神経芽細胞腫における遺伝子発現量と、予後良好型および予後不良型の臨床組織における同一遺伝子の遺伝子発現量を比較すると、4 s 期神経芽細胞腫において特異的である(すなわち、いずれよりもかなり高いか、或いはかなり低い)。従って、これらの遺伝子の少なくともひとつの存在を臨床組織サンプルに検出すれば、4 s 期神経芽細胞腫である可能性が高いとの判定ができる。

【0075】 また、グループ VII に属する遺伝子は、4 s 期神経芽細胞腫の臨 床組織においてのみ、検出されている。従って、この遺伝子の存在を臨床組織サ ンプルに検出すれば、4 s 期神経芽細胞腫である可能性が高いとの判定ができる ことになる。

【0076】 さらに、残りのグループに属する遺伝子群も、4 s 期神経芽細胞 腫における、遺伝子発現量と、予後良好型および予後不良型の臨床組織における 同一遺伝子の遺伝子発現量を比較すると、上記のような発現パターンが見出される。従って、これらの遺伝子の発現パターンを複数個、検出して、それらを解析 すれば、検定する臨床組織サンプルが4 s 期神経芽細胞腫であるかどうかの判定 ができる。特に、この目的で本発明の核酸を使用するとき、後述の核酸マイクロアレイを作製して、前記判定に供することが好ましい。

【0077】 このように、本発明の核酸は神経芽細胞腫の予後の良不良を診断する腫瘍マーカーとして有用である。すなわち、本発明は、ヒト神経芽細胞腫の予後およびそれに関連する様々な遺伝子情報を以下の手段により提供可能とする。

【0078】 (1) ハイブリダイゼーションに用いるプローブ

本発明の1つの実施の形態に従えば、本発明の核酸をハイブリダイゼーションのプローブ (すなわち、本発明の核酸プローブ) として使用することによって、神経芽細胞腫で発現している本発明の遺伝子を検出することが可能である。 さらに、本発明の核酸をハイブリダイゼーションのプローブとして使用し、様々な腫

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瘍、正常組織における遺伝子発現を調べることによって、該遺伝子発現の分布を 同定することも可能である。

【0079】 本発明の核酸をハイブリダイゼーションのプロープとして使用する場合、ハイブリダイゼーション方法自身については特に限定されない。好適な方法としては、例えばノザンハイブリダイゼーション、サザンハイブリダイゼーション、コロニーハイブリダイゼーション、ドットハイブリダイゼーション、Fluorescence in situ hybridization (F I S H)、in situ hybridization (I S H)、DNAチップ法、マイクロアレイ法、などが挙げられる。

【0080】 前記ハイブリダイゼーションの1つの応用例として、本発明の核酸をノザンハイブリダイゼーションのプローブとして用い、検定する臨床組織サンプル中においてmRNAの長さを測定することや、遺伝子発現を定量的に検出することが可能である。

【0081】 また、別の応用例として、本発明の核酸をサザンハイブリダイゼーションのプロープとして用い、検定する臨床組織サンプルのゲノムDNA中の、該DNA配列の有無を検出することが可能である。

【0082】 さらに別の応用例として、本発明の核酸をFISH法のプローブとして用い、本発明の遺伝子の染色体上の位置を同定することも可能である。

【0083】 さらに別の応用例として、本発明の核酸をISH法のプローブとして用い、本発明の遺伝子の発現の組織分布を同定することも可能である。

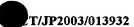
【0084】 本発明の核酸をハイブリダイゼーション用プロープとして使用する場合、少なくとも20個の塩基長が必要であり、本発明の核酸のうち、20個以上の連続した塩基からなる核酸が好ましく用いられる。より好ましくは、40個以上の連続した塩基からなる核酸が用いられる。特に好ましくは、60個以上の連続した塩基からなる核酸が用いられる。さらに、配列表の配列番号1~174に記載の核酸配列の全長からなる核酸を用いてもよい。

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当業者にとって、上記各種のハイプリダイゼーションにおける核 酸プローブ技法は周知であり、例えば、個々の塩基長を有する本発明の核酸プロ ープと、目的とするポリヌクレオチドとの適当なハイプリダイズ条件は容易に決 定することができる。種々の塩基長を含むプローブに対し至適であるハイブリダ イズ条件を得るためのかかる操作は、当業者では周知であり、例えば、Sambrook ら、Molecular Cloning: A laboratory manual (前掲) を参照して、行えばよい。 好ましくは、本発明の核酸プローブは、容易に検出されるように [0086] 標識される。検出可能な標識は、目視によって、または機器を用いるかのいずれ かによって検出され得るいかなる種類、元素または化合物であってもよい。通常 使用される検出可能な標識としては、放射性同位元素、アビジンまたはビオチン、 蛍光物質(FITCまたはローダミン等)が挙げられる。前記放射性同位元素は、 ³²P、¹⁴C、¹²⁵I、³H、³⁵S等である。また、ビオチン標識ヌクレオチドは、ニッ クトランスレーション、化学的または酵素的手段によって、核酸に組み込むこと ができる。ビオチン標識されたプローブは、アビジン/ストレプトアビジン、蛍 光標識、酵素、金コロイド複合体等などの標識手段を使用したハイブリダイゼー ション後に検出される。また、本発明の核酸プローブは、タンパク質と結合させ ることによって標識されてもよい。その目的で、例えば放射性または蛍光ヒスト ン一本鎖結合タンパク質が使用される。このようにして、適当に標識されたプロ ーブは、本発明の診断剤を構成する。

【0087】 (2) PCRに用いるプライマー

本発明の遺伝子を検出するには上記のハイブリダイゼーション法の他に、本発明の核酸に含まれる任意の核酸(DNA)配列からプライマーを設計して、Polymerase Chain Reaction (PCR) 法を用いることにより可能である。例えば、検定する臨床組織サンプルからmRNAを抽出し、RT-PCR法により遺伝子発現を半定量的に測定することが可能である。このような方法は、当業者にとって周知の方法に従って行われるが、例えば、Sambrook ら、Molecular Cloning: A

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laboratory manual (前掲)、および遺伝子病入門(高久史麿著:南江堂)が参照される。

【0088】 本発明の核酸 (DNA) をPCR用プライマー (すなわち、本発明のプライマー)として使用する場合、10ないし60個の塩基長が必要であり、本発明に係る核酸配列の一部であって、10ないし60個の連続した塩基を有する核酸が好ましく用いられる。より好ましくは、15ないし30個の塩基を有するものが用いられる。また一般的には、プライマー配列中のGC含量が40ないし60%のものが好ましい。さらに、増幅に用いる2つのプライマー間のTm値に差がないことが望まれる。また、プライマーの3'末端でアニールせず、プライマー内で2次構造をとらないことも望ましい。

【0089】 (3) 遺伝子のスグリーニング

本発明の核酸を使用することによって、神経芽細胞腫のみならず様々な組織や 細胞で発現している本発明の遺伝子の発現(またはその分布)を検出することが 可能である。これは例えば、本発明の核酸を上記のようにハイブリダイゼーショ ンのプローブ、またはPCRのプライマーとして使用することによって、可能と なる。

【0090】 また、DNAチップ、核酸マイクロアレイ等を用いても遺伝子の発現分布を検出することが可能である。すなわち、本発明の核酸を直接、前記チップ、アレイ上に張り付けることが出来る。チップ、アレイに張り付けるために、高精度分注機でかかる核酸等(DNA)を基板にスポットする方法が知られている(例えば、米国特許第5807522号を参照)。そこに臨床組織サンプルから抽出したmRNAを蛍光物質などで標識し、ハイブリダイズさせ、その遺伝子がどの様な組織の細胞で高発現しているかを解析することが可能である。またチップ、アレイ上に張り付けるDNAは、本発明の核酸またはその断片をプローブとして用いたPCRの反応産物であってもよい。別法として、本発明の核酸断片

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(DNA断片) を基板上で直接合成してDNAチップ若しくはアレイとすること もできる(例えば、米国特許第5424186号を参照)。

【0091】 (4) DNAのクローニング

本発明の核酸を使用することによってヒト神経芽細胞腫において発現している遺伝子をクローニングすることが可能である。例えば、本発明の核酸をノザンハイブリダイゼーションのプローブ、コロニーハイブリダイゼーションのプローブまたはPCRのプライマーとして使用し、本発明の遺伝子をクローニングすることが可能である。クローニング可能な遺伝子としては特に、予後不良型の神経芽細胞腫と予後不良型の神経芽細胞腫で発現量に差がある遺伝子、4s期神経芽細胞腫で発現する遺伝子、他の組織や癌細胞での発現様式とは異なって発現している遺伝子、細胞周期依存的に発現している遺伝子、神経分化に伴って誘導される遺伝子、癌遺伝子または癌抑制遺伝子によって発現が制御される遺伝子等が挙げられる。

【0092】 (5) 腫瘍の予後診断の方法およびそのために使用可能な腫瘍マーカー

上述のように本発明の遺伝子は、4 s 期神経芽細胞腫(予後良好型および予後不良型の神経芽細胞腫を含めて)において発現が見出された。そこで、本発明の核酸をハイブリダイゼーションのプロープ或いはPCRのプライマーとして使用し、被験者から採取した、検定する臨床組織サンプル中で、前記遺伝子の発現パターンを調べることにより予後診断(4 s 期神経芽細胞腫の判定)が行える。遺伝子の検出方法としては、前述のノーザンブロットハイブリダイゼーション法、インサイチュハイブリダイゼーション法、およびRT-PCR法等が挙げられる。【0093】 ハイブリダイゼーション法を用いるとき、検出する臨床組織サンプル中で前記核酸プローブとハイブリダイズする核酸の量を対照サンプル(例えば、予後良好型および予後不良型の神経芽細胞腫からの臨床組織)と比較して、遺伝子発現パターンを決定する。このようにして遺伝子発現パターンを検出する

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のに使用したそれぞれの核酸について、例えば、表1に記載の発現パターンと比較、解析して、予後診断できる。この目的では、前記の核酸マイクロアレイの使用が望ましい。また、RTーPCR法を用いるとき、サンプルからmRNAを抽出し、これをDNAに逆転写して、前記プライマーにより増幅するRTーPCR法を用いて、遺伝子発現を半定量的に測定する。それから前記と同様にして、予後診断できる。この目的のためには、該プライマーを必須成分として一組含有する診断キットを用いることが好ましい。該診断キットは、プライマー成分以外に、PCR用の緩衝液、洗浄液、および酵素等の公知の成分を含む。

【0094】 (6) アンチセンスオリゴヌクレオチド

本発明の別の実施の形態に従えば、本発明の核酸に対するアンチセンスオリゴヌクレオチドが提供される。前記アンチセンスオリゴヌクレオチドは、本発明の核酸にハイブリダイズすることが可能であり、アンチセンスDNAとアンチセンスRNAとを含む。アンチセンスDNAは、DNAからmRNAへの転写を阻害し、アンチセンスRNAは、mRNAの翻訳を阻害する。このようなアンチセンスオリゴヌクレオチドは、自動合成機を使用して、または本発明の核酸を鋳型とするPCR法により合成できる。さらに、該アンチセンスオリゴヌクレオチドは、DNAやmRNAとの結合力、組織選択性、細胞透過性、ヌクレアーゼ耐性、細胞内安定性が高められたアンチセンスオリゴヌクレオチド誘導体をも包含する。このような誘導体は、公知のアンチセンス技術を用いて、合成することができる。

【0095】 mRNAの翻訳開始コドン付近、リボソーム結合部位、キャッピング部位、スプライス部位の配列に相補的な配列を有するアンチセンスオリゴヌクレオチドは、該RNAの合成を阻止することができ、特に遺伝子の発現抑制効果が高い。従って、本発明は、かかるアンチセンスオリゴヌクレオチドを好適に包含する。

25 【0096】 (7)遺伝子治療

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本発明の別の実施の形態に従えば、遺伝子治療に用いられる治療用遺伝子をコードする核酸配列が提供される。そこで、本発明の核酸を遺伝子運搬に使用されるベクターに導入して、任意の発現プロモーターにより導入遺伝子(本発明の遺伝子)を発現させ、遺伝子治療に用いることができる。

【0097】 1. ベクター

導入されうるウイルスベクターは、DNAまたはRNAウイルスをもとに作製 できる。このようなベクターは、MoMLVベクター、ヘルペスウイルスベクタ ー、アデノウイルスベクター、AAVベクター、HIVベクター、SIVベクタ ー、センダイウイルスベクター等のいかなるウイルスベクターであってもよい。 また、ウイルスベクターの構成タンパク質群のうち1つ以上を、異種ウイルスの 構成タンパク質に置換する、または、遺伝子情報を構成する核酸配列のうち一部 を異種ウイルスの核酸配列に置換する、シュードタイプ型のウイルスベクターも 本発明に使用できる。例えば、HIVの外皮タンパク質であるEnvタンパク質 を、小水痘性口内炎ウイルス(vesicular stomatitis Virus: VSV)の外皮タンパ ク質であるVSV-Gタンパク質に置換したシュードタイプウイルスベクターが 挙げられる (Naldini L., Science, U.S.A., 1996, Vol. 272, p. 263)。 さら に、治療効果を持つウイルスであれば、ヒト以外の宿主域を持つウイルスもウイ ルスベクターとして使用可能である。ウイルス以外のベクターとしてはリン酸カ ルシウムと核酸の複合体、リポソーム、カチオン脂質複合体、センダイウイルス リポソーム、ポリカチオンを主鎖とする高分子キャリアー等が使用可能である。 さらに遺伝子導入系としてはエレクトロポレーション、遺伝子銃等も使用可能で ある。

【0098】 2. 発現プロモーター

さらに、治療用遺伝子に用いられる発現カセットは、標的細胞内で遺伝子を発 現させることができるものであれば、特に制限されることなくいかなるものでも 用いることができる。当業者はそのような発現カセットを容易に選択することが

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できる。好ましくは、動物由来の細胞内で遺伝子発現が可能な発現カセットであり、より好ましくは、哺乳類由来の細胞内で遺伝子発現が可能な発現カセットであり、特に好ましくは、ヒト由来の細胞内で遺伝子発現が可能な発現カセットである。発現カセットに用いられる遺伝子プロモーターは、例えばアデノウイルス、サイトメガロウイルス、ヒト免疫不全ウイルス、シミアンウイルス40、ラウス肉腫ウイルス、単純ヘルペスウイルス、マウス白血病ウイルス、シンビスウイルス、A型肝炎ウイルス、B型肝炎ウイルス、C型肝炎ウイルス、パピローマウイルス、ヒトT細胞白血病ウイルス、インフルエンザウイルス、日本脳炎ウイルス、JCウイルス、パルボウイルスB19、ポリオウイルス等のウイルス由来のプロモーター、アルブミン、SR α、熱ショック蛋白、エロンゲーション因子等の哺乳類由来のプロモーター、CAGプロモーター等のキメラ型プロモーター、テトラサイクリン、ステロイド等によって発現が誘導されるプロモーターを含む。

【0099】 3. 医薬品

遺伝子治療に用いる医薬品は、上記のような治療用にデザインされた薬物遺伝子を含む組換えウイルスベクターとして調製される。より具体的に言えば、本発明の遺伝子を含む組換えウイルスベクターを、水、生理食塩水、等張化した緩衝液等の適当な溶媒に溶解することで調製できる。その際、ポリエチレングリコール、グルコース、各種アミノ酸、コラーゲン、アルブミン等を保護材として添加しても調製可能である。

20 【0100】 4. 投与法、投与量

上記医薬品の生体への投与の方法については特に制限はない。例えば非経口的投与(注射投与など)することにより好ましく実施できる。その医薬品の使用量は、その使用方法、使用目的等により異なり、当業者は容易に適宜選択および最適化することが可能である。例えば、注射投与して用いる場合には、1 日量約0. 1 μ g/k g~1,000 m g/k gを投与するのが好ましく、より好ましくは、1 日量約1 μ g/k g~100 m g/k g である。

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【0101】 以下、実施例に即してさらに詳しく説明するが、本発明の技術的範囲はこれらの例に限定されるものではない。

【0102】 (実施例)

以下、実施例に基づいて本発明をより具体的に説明するが、本発明は以下の実施例に限定されるものではない。

【0103】 (製造例1) 神経芽細胞腫からのcDNAライブラリーの作製 1. サンプル入手

ヒト神経芽細胞腫(4 s 期)の臨床組織サンプルを手術摘出直後に準無菌的に 凍結し、その後-80℃に保存した。

【0104】 2. mRNAの調製

1に記載のサンプル2~3gを Total RNA Extraction Kit (QIGEN 社製) で処理し、トータルRNAを抽出した。抽出したトータルRNAをオリゴ d Tセルロースカラム (Collaborative 社製) を用いて、poly A構造を有するmRNAプールに精製した。さらに、以下の手順に従い、オリゴキャッピング法(Y.

15 Suzuki et al., Gene, U.S.A., 1997, Vol. 200, pp. 149-156)を用いてcDN Aライプラリーを調製した。

【0105】 3. mRNAの脱リン酸化

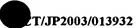
上記 2 において調製した $100 \sim 200 \mu$ g の m R N A プールを 67.3μ 1 の 0.1% ジエチルピロカーボネート(DEPC)を含む滅菌超純水(DEPC ー H_2O)に溶解させ、 20μ $105 \times BAP$ バッファー [TrisーHC1(50 mM、pH=7.0)/メルカプトエタノール(50 mM)]、 2.7μ 1 の R N a sin(40 unit/ μ 1:Promega社製)、 10μ 1 の B AP(0.25 unit/ μ 1、バクテリア由来アルカリフォスファターゼ:宝酒造社製)を加えた。この混合液を 37%で1時間反応させ、m R N Aの 5% 末端の脱リン酸化処理を行った。その後、フェノール・クロロホルム処理を 20% に以り、 最後にエタノール沈殿により、 脱リン酸化m R N Aプールを精製した。

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【0106】 4. 脱リン酸化mRNAの脱キャップ処理

上記3において調製した脱リン酸化mRNAプールの全量を $75.3\mu100.1\%$ DEPCを含む滅菌超純水に溶解させ、 $20\mu105$ xTAPバッファー[酢酸ナトリウム (250mM、pH=5.5) /メルカプトエタノール (50mM)、EDTA (5mM、pH=8.0)]、 $2.7\mu10$ RNasin (40unit/ $\mu1)$ 、 $2\mu10$ TAP (10Dacco Acid pyrophosphatase: 20unit/ $\mu1$)]を加えた。この混合液を37で1時間反応させ、脱リン酸化mRNAの5、末端の脱キャップ処理を行った。この際、キャップ構造を持たない不完全長の脱リン酸化mRNAは、脱キャップ処理されず5、末端は脱リン酸化された状態に留まった。その後、フェノール・クロロホルム処理、エタノール沈殿により、脱キャップmRNAプールを精製した。

【0107】 5. オリゴキャップmRNAの調製

上記4において調製した脱キャップmRNAプールの全量を $11\mu100$. 1%DEPCを含む滅菌超純水に溶解させ、 $4\mu105$ 'ーオリゴRNA(5'ーAGCAUCGAGUCGGCCUUGGCCUACUGG-3':配列番号1079; $100ng/\mu1$)、 $10\mu10010x1$ igationバッファー[Tris-HC1(500mM、pH=7.0)/メルカプトエタノール(100mM)]、 $10\mu100$ 塩化マグネシウム(50mM)、 $2.5\mu100$ ATP(24mM)、 $2.5\mu100$ RNasin ($40unit/\mu1$)、 $10\mu100$ T4 RNA 1igase ($25unit/\mu1$: 室酒造社製)、 $50\mu100$ ポリエチレングリコール(50%w/v、PEG8000: シグマ社製)を加えた。この混合液を20%v3時間反応させ、脱キャップmRNAの5'末端に5'ーオリゴRNAを連結した。この際、キャップ構造を持たない不完全長の脱リン酸化mRNAは、5'ーオリゴRNAが連結されない。その後、フェノール・クロロホルム処理、エタノール沈殿により、オリゴキャップmRNAプールを精製した。

【0108】 6. オリゴキャップmRNAからのDNA除去

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上記5において調製したオリゴキャップmRNAプールを70.3 μ 1の0.1%DEPCを含む滅菌超純水に溶解させ、 4μ 1のTrisーHC1 (1M、pH=7.0)、5.0 μ 1のDTT (0.1M)、16 μ 1の塩化マグネシウム (50mM)、2.7 μ 1のRNasin (40 μ 1)、2 μ 1のDNaseI (5 μ 1)、2 μ 1のDNaseI (5 μ 1)、2 μ 1のDNaseI (5 μ 1)、2 μ 1の で10分間反応させ、余分なDNAを分解した。その後、フェノール・クロロホルム処理、エタノール沈殿、カラム精製(S-400HR:ファルマシアバイオテック社製)により、DNA (-) オリゴキャップmRNAプールを精製した。【0109】 7. First Strand c DNAの調製

上記6において調製したDNA(-)オリゴキャップmRNAプールを、Super Script II (ライフテックオリエンタル社製キット)を用いて逆転写し、First Strand c DNAプールを得た。

【0111】 8. Second Strand c DNAの調製

上記7において調製した First Strand c DNAプールを、Gene Amp (パーキンエルマー社製キット)を用いて、PCR増幅した。First Strand c DNAプールを $52.4\mu1$ の滅菌蒸留水に溶解させ、 $30\mu1$ の3.3xRe a c t i o n バッファー(キット付属品)、 $8\mu1$ のd NTP m i x (2.5

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mM、キット付属品)、4. 4μ 1の酢酸マグネシウム(25mM、キット付属品)、1. 6μ 1のプライマーF(10pmo1/ μ 1、5'ー

AGCATCGAGTCGGCCTTGTTG - 3'配列番号 1 0 8 1)、1. 6 μ 1 のプライマーR(1 0 p m o 1 / μ 1、5' -GCGCTGAAGACGGCCTATGT - 3'配列番号 1 0 8 2)、2 μ 1 の r T t h(キット付属品)を加えた。この混合液に、1 0 0 μ 1 のミネラルオイルを静かに加え重層した。この反応液を 9 4 $\mathbb C$ で 5 分間変性させた後、9 4 $\mathbb C$ 、1 分間、5 2 $\mathbb C$ 、1 分間、7 2 $\mathbb C$ 、1 0 分間を 1 サイクルとして 1 2 サイクル繰り返し、さらに 7 2 $\mathbb C$ で 1 0 分間放置し、1 P C R 反応を行った。その後、フェノール・クロロホルム処理、エタノール沈殿で精製し、Second Strand 1 C D N A 1 ールを得た。

【0112】 9. Second Strand cDNAのSfil処理

上記8において調製した Second Strand $cDNAプールを87\mu1の滅菌蒸留水に溶解させ、<math>10xNEB$ バッファー (NEB社製)、100xBSA(ウシ血清アルプミン、NEB社製)、 $2\mu1$ のSfiI(制限酵素、20unit $/\mu1$ 、NEB社製)を加えた。この混合液を50で一晩反応させ、SfiI による制限酵素処理を行った。その後、フェノール・クロロホルム処理、エタノール沈殿で精製し、両末端がSfiI 処理されたcDNAプールを得た。

【0113】 10. SfiI処理されたcDNAのサイズ分画

【0114】 11. cDNAライブラリー

上記10において調製した長鎖cDNAプールを DNA Ligation kit ver.1(宝酒造社製キット)を用いてクローニングベクターであるpME18S-FL3(東

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京大学医科学研究所 菅野純夫教授より供与)にライゲーションを行った。長鎖 cDNAプールを 8μ 1の滅菌蒸留水に溶解し、あらかじめ制限酵素DraII1で処理した 1μ 1のpME18S-FL3、80 μ 1のSolution4(キット付属品)、10 μ 1のSolution8(キット付属品)を加え、16 $\mathbb C$ で3時間反応させた。その後、フェノール・クロロホルム処理、エタノール沈殿で精製しcDNAライブラリーを得た。

【0115】 (実施例1)大腸菌へのトランスフォーメーション

1. クローニング

製造例1012で調製したcDNAライブラリーを大腸菌(TOP-10、 Invitrogen 社製)にトランスフォーメーションした。すなわち、cDNAライブ ラリーを $10\mu1$ の滅菌蒸留水に溶解し、TOP-10に混合した。その後、氷上にTCOP-10に混合した。その後、氷上にTCOP-10に混合した。その後、氷上にTCOP-10に混合した。TCOP-10に混合した。その後、氷上にTCOP-10に混合した。その後、氷上にTCOP-10に混合した。TCOP-10に混合した。TCOP-10に混合した。TCOP-10に混合した。TCOP-10に表した。T

【0116】 2. 大腸菌クローンの保存(グリセロールストックの調製)

上記1において得られた寒天培地上の各大腸菌クローンを、爪楊枝にて拾い上げ、96穴プレートに準備した $120\mu1$ のLB培地中に懸濁させた。この96穴プレートを37℃で一晩静置し、大腸菌の培養を行った。その後、60%グリセロール溶液を $72\mu1$ 加え、-20℃で保存した(グリセロールストック)。

【0117】 (実施例2)核酸配列決定

1. プラスミドの調製

実施例1の2で調製した $10\mu1$ のグリセロールストックを15m1の遠心チューブに移し、3m1のLB培地、 50μ g/m1のアンピシリンを加え、 37° C で一晩振盪し、大腸菌の培養を行った。その後、QIA Prep Spin Miniprep Kit (QIAGEN 社製)を用いて大腸菌からプラスミドDNAを抽出、精製した。

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【0118】 2. 両末端シークエンスの解析

上記1において調製したプラスミドDNAを DNA Sequencing Kit(ABI社製キット)を用いて両末端のシークエンスを決定した。600ngのプラスミドDNA、 $8\mu1$ のプレミックス(キット付属品)、3.2pmo1のプライマーを混合し、滅菌蒸留水で合計 $20\mu1$ になるように調製した。この混合液を96%で2分間変性させた後、96%、10秒間、<math>50%、5秒間、<math>60%、4分間を1サイクルとして<math>25サイクル繰り返し反応を行った。その後エタノール沈殿で精製した。変性条件下でポリアクリルアミドゲルにて電気泳動を行い、ABI377 (ABI社製)を用いて配列決定を行った。

【0119】 (実施例3)データベースを用いるホモロジー検索

実施例2において両末端シークエンスを解析して得られたサンプルのDNA配列情報についてインターネットを介したDNA配列のホモロジー検索を行った。 検索にはNCBI (National Center of Biotechnology Information USA, http://www.ncbi.nlm.nih.gov/BLAST)のBLASTを用いた。BLASTサーチのソフトとして、DYNACLUST Ver. 4.0 (DYNACOM社)を使用した。ホモロジー検索の結果、約2700個の遺伝子を同定した。これらの遺伝子を分類し、RepeatMasker ソフトを使用して反復配列を取り除いたところ、1598個の遺伝子が得られた。そのうち、新規な遺伝子は、963個であり、既知の遺伝子は635個であった。

【0120】 これらの遺伝子のうち、新規なもの308個については、シークエンスできたもの関して、配列表にそれらの部分解読配列を示してある。

【0121】 (実施例4) 半定量的RT-PCRによる遺伝子発現の比較 1. サンプル入手

ヒト神経芽細胞腫(4 s 期)の臨床組織サンプルを手術摘出直後に準無菌的に 凍結し、その後-80℃に保存した。このようなサンプルを8検体用意した。同



様に、予後良好型および予後不良型のヒト神経芽細胞腫の臨床組織サンプルを各 12検体づつ用意した。

【0122】 予後良好型および予後不良型の神経芽細胞腫サンプルについては、 予後の検定を以下の指標をもとに行ったものである。

5 予後良好型:

- 病期1または2
- ・発症年齢が1歳未満
- ・手術後5年以上再発なく生存
- ・N-mycの増幅なし

10 予後不良型:

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- · 病期 4
- ・発症年齢が1歳以上
- ・手術後3年以内に死亡
- ·N-myc増幅あり
- 15 【0123】 2. ディファレンシャルスクリーニング 各検体の半定量的RT-PCRは以下の方法により実施した。

【0124】 a) 逆転写(RT) 反応

検体からのRNAを SuperScript II reverse transcriptase(GIBCO社製)を用いて、cDNAに逆転写した。すなわち、トータルRNA20 μ g、8 μ l のランダムプライマー(1 μ g/ μ l)(宝酒造社製)、および必要量のDEP Cを含む滅菌超純水で48 μ lの溶液を調製した。この溶液を65 $\mathbb C$ で15分間、インキュベートし、反応終了後氷上に置いた。24 μ lの5 x First Strand Buffer (GIBCO社製)、12 μ lの0.1M DTT (GIBCO社製)、30 μ lのdNTPs(宝酒造社製)、4 μ lの Super Script II reverse transcriptase、および2 μ lのDEPCを含む滅菌超純水を混合して、72 μ lの混合液を調製した。この混合液を前記の氷冷した溶液に加え、総量を120 μ lとし、42 $\mathbb C$

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で1.5時間、次いで95℃で5分間反応させた。これを-20℃で保存し、P CR鋳型の母液とした。

【0125】 このように調製したcDNA溶液をDDWで適当な倍率に希釈し、GAPDHプライマーを用いて、標準化(濃度調整)した。使用したGAPDHプライマーの塩基配列は、下記の通りであった。

5'-ACCTGACCTGCCGTCTAGAA-3' (forward:配列番号1077) 5'-TCCACCACCCTGTTGCTGTA-3' (reverse:配列番号1078)

【0126】 続いて、DDWで希釈、濃度調整した各サンプルを下記のPCR 反応に供した。

lo 【0127】 b) PCR反応

【0128】 このように設定した条件でPCRを行った産物を1.5%アガロースゲルで20分間電気泳動し、エチジウムブロミドで染色して、3組の検体(4s期神経芽細胞腫、予後良好型の神経芽細胞腫、および予後不良型の神経芽細胞腫)におけるバンドの濃度を比較した。

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【0129】 得られた発現パターンを検体サブセット間で、まとめたものが既 出の表1である。また、発現パターンの解析の結果は、既に議論した通りである。

【0130】 なお、使用したプライマーは、検出しようとする遺伝子の末端シークエンス(実施例3)を Primer3 ソフトに入力して、適当なプライマー選択条件(塩基数、Tm、GC%)で選定した。前出の特定クローンに対応するプライマー配列は、配列表(配列番号175~1076)に与えられている。

産業上の利用可能性

【0131】 以上説明したように、本発明の遺伝子または本発明の核酸から得られる情報を利用することにより、検定する臨床組織サンプルから該遺伝子を検出して、神経芽細胞腫の予後診断(主に4 s 期神経芽細胞腫の判定)が可能となる。具体的には、前記遺伝子若しくは核酸から得られる情報を腫瘍マーカーに利用することにより、予後診断に使用可能な、診断剤の調製或いは診断用核酸マイクロアレイを設計することが可能となる。

【0132】 4 s 期神経芽細胞腫の正しい診断ができれば、対象患者に治療が 必要かどうかの判断の重要な情報となり、場合によれば不必要な外科手術を避け ることができる。



請求の範囲

- 1. 配列表の配列番号1ないし174に記載の核酸配列からなる群より選ばれる1つの配列からなる核酸。
- 2. 配列表の配列番号1ないし14に記載の核酸配列からなる群より選ばれる 1つの配列からなる、請求項1に記載の核酸。
- 3. 請求項1または2に記載の核酸に相補的な核酸。
- 4. 請求項1ないし3のいずれか1項に記載の核酸と、ストリンジェントな条件下でハイブリダイズする核酸。
- 5. 以下の(a)或いは(b)の核酸を含む核酸プローブ:
- 10 (a)配列表の配列番号1ないし174に記載の核酸配列からなる群より選ばれる 1つの配列の全長若しくは一部からなる核酸、またはそれに相補的な核酸;
 - (b) 配列表の配列番号1ないし174に記載の核酸配列からなる群より選ばれる 1つの配列からなる核酸とストリンジェントな条件下でハイブリダイズする核酸 、またはそれに相補的な核酸。
- 15 6. 以下の(a)或いは(b)の核酸を含む請求項5に記載の核酸プローブ:
 - (a)配列表の配列番号1ないし14に記載の核酸配列からなる群より選ばれる1つの配列の全長若しくは一部からなる核酸、若しくはそれに相補的な核酸;
 - (b) 配列表の配列番号1ないし14に記載の核酸配列からなる群より選ばれる1 つの配列からなる核酸とストリンジェントな条件下でハイブリダイズする核酸、
- 20 若しくはそれに相補的な核酸。
 - 7. 請求項5または6に記載の核酸プローブを有効成分として含有する4s期神経芽細胞腫の診断剤。
 - 8. 以下の(a)或いは(b)のDNAを含むプライマー:
- (a) 配列表の配列番号175ないし1076に記載の核酸配列からなる群より選 25 ばれる1つの配列からなるDNA、またはそれに相補的なDNA;



- (b)配列表の配列番号175ないし1076に記載の核酸配列からなる群より選ばれる1つの配列からなるDNAとストリンジェントな条件下でハイブリダイズするDNA、またはそれに相補的なDNA。
- 9. 以下の(a)或いは(b)のDNAを含むプライマー:
- 5 (a)配列表の配列番号175ないし202に記載の核酸配列、および配列番号519ないし540に記載の核酸配列からなる群より選ばれる1つの配列からなるDNA、若しくはそれに相補的なDNA、または配列表の配列番号785ないし798に記載の核酸配列からなる群より選ばれる1つの配列からなるDNA、若しくはそれに相補的なDNA;
- 10 (b) 配列表の配列番号175ないし202に記載の核酸配列、および配列番号5 19ないし540に記載の核酸配列からなる群より選ばれる1つの配列からなる DNAと、または配列表の配列番号785ないし798に記載の核酸配列からな る群より選ばれる1つの配列からなるDNAとストリンジェントな条件下でハイ ブリダイズするDNA、若しくはそれに相補的なDNA。
- 10. 請求項8または9に記載のプライマーを一組、有効成分として含有する 4 s 期神経芽細胞腫の診断キット。
 - 11. 神経芽細胞腫の臨床組織サンプルから配列表の配列番号1ないし14に記載の核酸配列からなる群より選ばれる1つの配列からなる核酸の有無を検出することを特徴とする、4s期神経芽細胞腫の判定方法。
- 20 12. 固相支持体に、配列番号1ないし174に記載の核酸配列の全長若しくは一部からなる核酸を複数個組み合わせ、それらを固定してなる核酸マイクロアレイ。
- 13. 固相支持体に、配列番号175ないし202に記載の核酸配列、配列番号519ないし540に記載の核酸配列、および配列番号785ないし798に
 25 記載の核酸配列からなる核酸を複数個組み合わせ、それらを固定してなる核酸マイクロアレイ。



SEQUENCE LISTING

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<170> PatentIn Ver. 2.1

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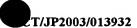
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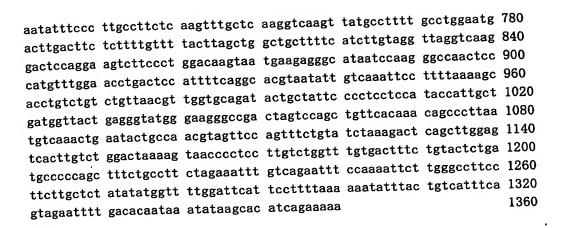
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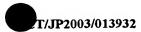
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WO 2004/039975



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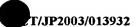
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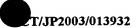
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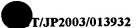
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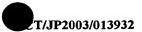
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- <213> Homo sapiens
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<213> Homo sapiens

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<223> nbla23339

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<213> Homo sapiens

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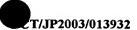
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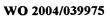
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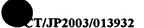
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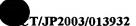
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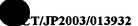
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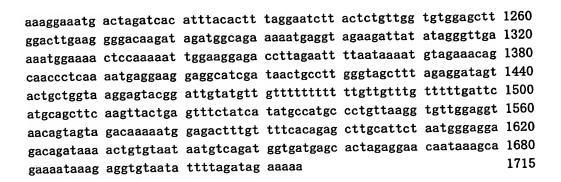
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<213> Homo sapiens

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<223> nbla23436

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- <213> Homo sapiens

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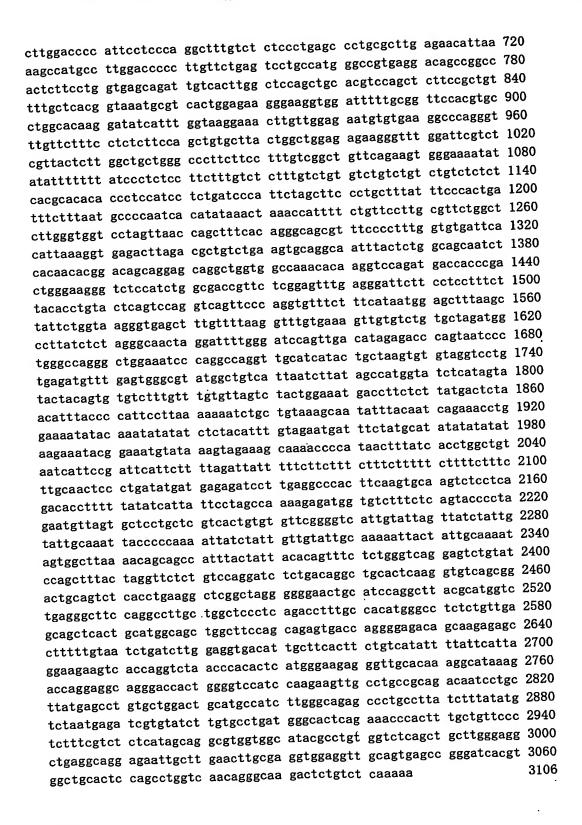
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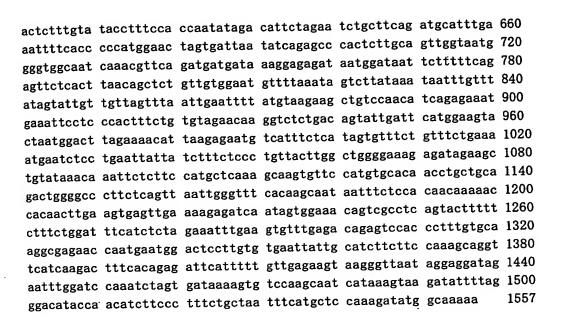
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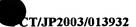
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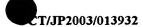


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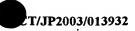
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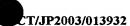
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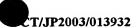
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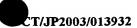
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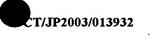


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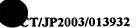
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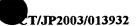
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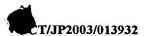




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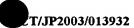
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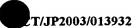
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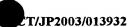
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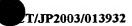
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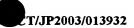
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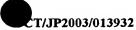
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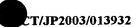
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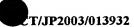
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ctgcgggttg gtaattgag 19

<210> 1053

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Primer: nbla21917-1f(k)

<400> 1053

tgagttctgg attgcctgtg 20

<210> 1054

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Primer: nbla21917-1r

<400> 1054

cagggcatgg attetttet 20

<210> 1055

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Primer: nbla22099-1f(k)

<400> 1055

ctggttccca cgcaagtaag 20

<210> 1056

⟨211⟩ 20	
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<210> 1057	•
<211> 20	
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(400) 1057	
<400> 1057	20
agcaggcatg gcaattttag	20
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<212> DNA	
<213> Artificial Sequence	
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<400> 1058	
ccagaggtgc agagaagtgt g	21
<210> 1059	•
<211> 21	
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Cado, Officiolio II Imel., Holdadado II (h)	•
<400> 1061	
tggtctcctt cctgtgttcc	20
<210> 1062	
⟨211⟩ 19	
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<pre><213> Artificial Sequence '</pre>	
THE PRESENCE OF THE PROPERTY O	
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<213> Artificial Sequence

<220>

_	
⟨400⟩ 1063	
acaagtccac accacagcac	20
acaag too ac accade accade accade accade too accade too accade ac	
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<210> 1065	
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⟨211⟩ 19	
<212> DNA	
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(220) Synthetic IIImii noiddibio II	
<400> 1066	
aaccacactc ctgcctcca	19
<210> 1067	
<211> 23	
<212> DNA	



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<210> 1070 <211> 21 <212> DNA <213> Artificial Sequence

<220>
<223> Synthetic Primer: nbla24871-1r
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21





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<211> 19	
<212> DNA (213) Artificial Servers	
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•	
<400> 1072	
catgctgttc tgcttgtgg	19
(010) 1070	
<210> 1073 <211> 22	
<212> DNA	
<pre><213> Artificial Sequence</pre>	
varov in tillicial dequence	
⟨220⟩	
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gagagcagcg attaaccaaa ag	22
<210> 1074	
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<220> <223> Synthetic Primer: nbla23300-1r

<400> 1074 acatcaac ttccctccaa 20

<210> 1075 <211> 21



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20

<2	10	\	ת	N.	٨
N۷	12	_	U.	EN.	н

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<400> 1075

ctttcatttc tcctgctgtc c

<210> 1076

<211> 22

<212> DNA

<213> Artificial Sequence

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<223> Synthetic Primer: nbla23664-1r

⟨400⟩ 1076

gggactcacc cattttctat tt 22

<210> 1077

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<212> DNA

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<400> 1077

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<223> Synthetic Primer: GAPD reverse

<400> 1078

tccaccaccc tgttgctgta

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agcatcgagt cggccttgtt g	21
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<pre><223> Synthetic Primer: reverse</pre>	
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gcgctgaaga cggcctatgt	20



International application No.
PCT/JP03/13932

	FICATION OF SUBJECT MATTER C1 ⁷ C12N15/09, C12Q1/68, G01N33	3/50		
According to	According to International Patent Classification (IPC) or to both national classification and IPC			
	SEARCHED			
Minimum do	cumentation searched (classification system followed b	y classification symbols)		
	CI CIZNIS/US, CIZQI/66, GUINS.	3/ JU		
	on searched other than minimum documentation to the			
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) GenBank/EMBL/DDBJ/Geneseq, WPI (DIALOG), BIOSIS (DIALOG), JSTPlus (JOIS), MEDLINE (STN)				
C. DOCUI	MENTS CONSIDERED TO BE RELEVANT			
Category*	Citation of document, with indication, where app		Relevant to claim No.	
А	Eggert, A. et al., High-level angiogenic factors is associa tumor stage in human neurobla Cancer Res., Vol.6, No.5, pag (2000)	ted with advanced stomas., Clin	1-13	
A	Gallego, S. et al., Differential polymerase chain reaction with serial dilutions for quantification of MYCN gene amplification in neuroblastoma., Anticancer Res., Vol.18, No.2A, pages 1211 to 1215, (1998)		1-13	
× Furth	er documents are listed in the continuation of Box C.	See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date or considered to be of particular relevance "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed Date of the actual completion of the international search 26 November, 2003 (26.11.03) "T" later document published after the international filing date of priority date and not in conflict with the application but cite understand the priority date and not in conflict with the application but cite understand the priority date and not in conflict with the application but cite understand the priority date and not in conflict with the application but cite understand the priority date and not in conflict with the application but cite understand the priority date and not in conflict with the application but cite understand the priority date and not in conflict with the application but cite understand the priority date and not in conflict with the application but cite understand the priority date and not in conflict with the application but cite understand the priority date and not in conflict with the application but cite understand the priority date and not in conflict with the application but cite understand the priority date and not in conflict with the application document of particular relevance; the claimed invention can considered to involve an inventive step when the document of particular relevance; the claimed invention can considered to involve an inventive step when the document of particular relevance; the claimed invention can considered to involve an inventive step when the document of partic		the application but cited to derlying the invention cannot be cred to involve an inventive in claimed invention cannot be cred to involve an inventive in claimed invention cannot be powhen the document is he documents, such in skilled in the art it family		
Name and mailing address of the ISN Japanese Patent Office		Authorized officer		
Pageinile 3	la.	Telephone No		



INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP03/13932

C(Continua	tion). DOCUMENTS CONSIDERED TO BE RELEVANT	<u></u>	~
Cotonomia			
-arckory.	Citation of document, with indication, where appropriate, of the relev	ant passages	Relevant to claim No.
A	Shuichi YAMANE, "Shinkei Gashugun Shuyo n Saibo Shogaisei T Saibo ni yori Ninshiki Shuyo Taishuku Kogen no Idenshi Hatsugen' expression of tumor rejection antigens re by cytolytic T lymphocytes in neuroblasto related tumors), Journal of Kyoto Prefect University of Medicine, Vol.108, No.3, pages 381 to 388, (1999), particularly,	sareru ", (Gene ecognized oma- tural	1-13
A	The Sanger Center, et al., Toward a Comp. Human Genome Sequence., Genome Res., Vol No.11, pages 1097 to 1108 (1998), partice GenBank database Accession No.AC093879	.8,	1-6,8,9



INTERNATIONAL SEARCH REPORT

International application No. PCT/JP03/13932

Box I Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)			
This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:			
1. Claims Nos.:			
because they relate to subject matter not required to be searched by this Authority, namely:			
2. Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:			
3. Claims Nos.:			
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).			
Box II Observations where unity of invention is lacking (Continuation of item 3 of first sheet)			
This International Searching Authority found multiple inventions in this international application, as follows: (See extra sheet.)			
As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.			
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.			
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:			
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: The parts relating to SEQ ID NOS:1, 175 and 176 in claims.			
Remark on Protest The additional search fees were accompanied by the applicant's protest.			
No protest accompanied the payment of additional search fees.			



INTERNATIONAL SEARCH REPORT

International application No. PCT/JP03/13932

Continuation of Box No. II of continuation of first sheet(1)

The nucleic acids represented by SEQ ID NOS:1 to 174 and the primers represented by SEQ ID NOS:175 to 1076 as set forth in claims are common to each other exclusively in being usable in diagnosing and judging neuroblastoma at stage 4s.

However, specific genes having an expression pattern specific to neuroblastoma at stage 4s are reported by the following documents 1 to 3. Accordingly, diagnosis/judgment of neuroblastoma at stage 4s by using a specific gene having an expression pattern specific to neuroblastoma at stage 4s cannot be considered as a special technical feature in the meaning within PCT Rule 13.2.

According to PCT Rule 13.3, unity of inventions shall be determined without regard to whether the inventions are claimed in separate claims or as alternatives within a single claim.

Therefore, the inventions relating to the nucleic acids represented by SEQ ID NOS:1 to 174 and the primers represented by SEQ ID NOS:175 to 1076 are not considered as a group of inventions so linked as to form a single general inventive concept. Concerning the relationships among the nucleic acids represented by SEQ ID NOS:1 to 174 and primer pairs corresponding thereto (i.e., SEQ ID NOS:175 to 518 and SEQ ID NOS:1073 to 1076), unity of inventions is fulfilled for each nucleic acid (i.e., 174 groups of inventions) but the inventions relating to other primers represented by SEQ ID NOS:519 to 1072 are 554 independent groups of inventions. That is, it is recognized that claims of the present case have 728 groups of inventions in total.

- Document 1: Eggert, A. et al., High-level expression of angiogenic factors is associated with advanced tumor stage in human neuroblastomaomas.
 - Clin Cancer Res, Vol.6, No.5, pp. 1900-1908 (2000)
- Document 2: Gallego, S. et al., Differential polymerase chain reaction with serial dilutions for quantification of MYCN gene amplification in neuroblastomaoma. Anticancer Res, Vol.18, No.2A, pp. 1211-1215 (1998)
- Document 3: (Journal of Kotyo Prefectural University of Medicine), Vol.108, No.3, pp. 381-388 (1999)





国際出願番号 PCT/JP03/13932

A. 発明の属する分野の分類(国際特許分類(IPC)) Int. Cl ⁷ Cl2N15/09、Cl2Ql/68、G01N33/50			
B. 調査を行った分野 調査を行った最小限資料(国際特許分類(IPC)) Int. Cl' Cl2N15/09、Cl2Q1/68、G01N33/50			
最小限資料以外の資料で調査を行った分野に含まれるもの			
国際調査で使用した電子データベース(データベースの名称、調査に使用した用語) GenBank/EMBL/DDBJ/Geneseq、 WPI (DIALOG) 、BIOSIS (DIALOG) 、JSTPlus (JOIS) 、MEDLINE (STN)			
C. 関連・ 引用文献の	トると認められる文献		関連する
カテゴリー	メリカリ 引用文献名 及び一部の箇所が関連すると	きは、その関連する箇所の表示	請求の範囲の番号
A	Eggert, A. et al., High-level expression of angiogent th advanced tumor stage in human of Clin Cancer Res, Vol. 6, No. 5, pp. 196	neuroblastomas.	1-13
A	Gallego, S. et al., Differential polymerase chain read for quantification of MYCN gene at neuroblastoma. Anticancer Res, Vol. 18, No. 2A, pp. 12	mplification in	1-13
区欄の	続きにも文献が列挙されている。	□ パテントファミリーに関する別	紙を参照。
* 引用文献のカテゴリー 「A」特に関連のある文献ではなく、一般的技術水準を示すもの 「E」国際出願日前の出願または特許であるが、国際出願日 以後に公表されたもの 「L」優先権主張に疑義を提起する文献又は他の文献の発行日若しくは他の特別な理由を確立するために引用する文献(理由を付す) 「O」口頭による開示、使用、展示等に言及する文献「P」国際出願日前で、かつ優先権の主張の基礎となる出願「&」同一パテントファミリー文献		発明の原理又は理論 当該文献のみで発明 えられるもの 当該文献と他の1以 自明である組合せに	
国際調査を	完了した日 26.11.2003	国際調査報告の発送日 17.2	2. 2004
Ħ	関の名称及びあて先 本国特許庁 (ISA/JP) 郵便番号100-8915 京都千代田区霞が関三丁目4番3号	特許庁審査官(権限のある職員) 田村明照 電話番号 03-3581-1101	·





国際調査報告

国際出願番号 PCT/JP03/13932

	四队阿基林口		77 10 3 3 2
C(続き).	関連すると認められる文献		
引用文献の カテゴリー*	引用文献名 及び一部の箇所が関連するときは	、その関連する箇所の表示	関連する 請求の範囲の番号
A	山根秀一著、神経芽腫群腫瘍における細胞障害性T細胞に縮抗原の遺伝子発現 (Gene expression of antigens recognized by cytolytic T lymneuroblastoma-related tumors) 京府医大誌, Vol. 108, No. 3, pp. 381-388 (19	tumor rejection phocytes in	1-13
A	The Sanger Centre, et al., Toward a Complete Human Genome Sequence Genome Res., Vol. 8, No. 11, pp. 1097-1108 (1) 特にGenBank database Accession No. ACO93	1998),	1-6, 8, 9





国際出願番号 PCT/JP03/13932

第1欄 請求の範囲の一部の調査ができないときの意見(第1ページの2の続き) 法第8条第3項 (PCT17条(2)(a)) の規定により、この国際調査報告は次の理由により請求の範囲の一部について成しなかった。	作
1. 計求の範囲は、この国際調査機関が調査をすることを要しない対象に係るものである。 つまり、	
2. [] 請求の範囲は、有意義な国際調査をすることができる程度まで所定の要件を満たしていない国際出願の部分に係るものである。つまり、	`
3. [] 請求の範囲 は、従属請求の範囲であってPCT規則6.4(a)の第2文及び第3文の規定は 従って記載されていない。	٤
第Ⅱ欄 発明の単一性が欠如しているときの意見(第1ページの3の続き)	
次に述べるようにこの国際出願に二以上の発明があるとこの国際調査機関は認めた。	
(特別ページ参照)	
(特別へ一シ参照)	1
	l
	l
1. 出願人が必要な追加調査手数料をすべて期間内に納付したので、この国際調査報告は、すべての調査可能な話の範囲について作成した。	球
2. 〕 追加調査手数料を要求するまでもなく、すべての調査可能な請求の範囲について調査することができたので、 加調査手数料の納付を求めなかった。	追
3. 出願人が必要な追加調査手数料を一部のみしか期間内に納付しなかったので、この国際調査報告は、手数料の付のあった次の請求の範囲のみについて作成した。	つ納
4. 区 出願人が必要な追加調査手数料を期間内に納付しなかったので、この国際調査報告は、請求の範囲の最初に されている発明に係る次の請求の範囲について作成した。	己載
請求の範囲のうち配列番号1,175,176に関する部分	
追加調査手数料の異議の申立てに関する注意	
□ 追加調査手数料の納付と共に出願人から異議申立てがあった。 □ 追加調査手数料の納付と共に出願人から異議申立てがなかった。	
□ 地川明五十数杯ツ煮川(大に山田八川・り天成中立(かぶかつに。	





(第Ⅱ欄)

請求の範囲に記載された配列番号1-174に記載された核酸及び配列番号175乃至1076に記載されたプライマーは、4s期神経芽細胞腫の診断・判定に用いることができることにおいてのみ共通する。

しかしながら、下記文献1-3には、4 s 期の神経芽細胞腫において特異的な発現パターンを有する特定の遺伝子が記載されている。したがって、4 s 期の神経芽細胞腫において特異的な発現パターンを有する特定の遺伝子を用いて、4 s 期神経芽細胞腫の診断・判定を行うことは、PCT規則13.2における特別な技術的特徴であるとはいえない。

ここで、PCT規則13.3によると、発明の単一性の判断はこれらの発明が別個の請求 の範囲に記載されているか単一の請求の範囲に択一的な形式によって記載されているかを考 慮することなく行われるべきものである。

よって、請求の範囲に記載された発明のうち配列番号1-174に記載された核酸及び配列番号175乃至1076に記載されたプライマーに関する発明は、単一の一般的発明概念を形成するように連関している一群の発明であるとはいえない。そして、配列番号1-174に記載された核酸とそれに対応する一対のプライマー、すなわち配列番号175乃至518、1073乃至1076との関係においてはそれぞれの核酸ごと(174個の発明群)に単一性を満たすものの、配列番号519乃至1072に記載されたその他のプライマーに関する発明は個々に独立した554個の発明群であり、請求の範囲には併せて728個の発明群が記載されているものと認める。

文献1: Eggert, A. et al., High-level expression of angiogenic factors is associated with advanced tumor stage in human neuroblastomas. Clin Cancer Res. Vol. 6, No. 5, pp. 1900-1908 (2000)

文献 2: Gallego, S. et al., Differential polymerase chain reaction with serial dilutions for quantification of MYCN gene amplification in neuroblastoma. Anticancer Res. Vol. 18, No. 2A, pp. 1211-1215 (1998)

文献 3:京府医大誌, Vol. 108, No. 3, pp. 381-388(1999)